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WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM MORE THAN A YEAR OLD *

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In a previous report[1] I showed that glycerol is a suitable preservative for human serum intended for Wassermann reaction. Human serum was preserved for 3 months without any noticeable change in the result. This report deals with serum that has been preserved with glycerol for more than a year. As most of the specimens were reported on in the previous article, I shall arrange the tests and tables in accordance with the plan adopted at that time.

The Wassermann method with human hemolytic serum was used in this investigation.

As complement, the pooled serums of 2 or 3 guinea-pigs were used in quantities of 0.05, 0.025, and 0.0125 c.c.

The antigen was alcoholic extract of human heart muscle, and, as test dose, the largest quantity that was not anticomplementary in the antigen control was used. An antigen control was used with each lot of serum tested.

As hemolytic amboceptor, the serum of rabbits immunized with washed human blood corpuscles was used in doses of 1 and 1.25 units per tube.

The term unit was applied to the smallest quantity of hemolytic serum which, with 0.025 c.c. of complement, dissolved 0.25 c.c. of a 2.5% suspension of washed blood corpuscles in 1 hour. The total quantity in each tube was made up to 1.25 c.c., the same proportions, but only half the quantities used in the tests previously reported. The mixture of diluted amboceptor and corpuscles was allowed to stand at room temperature for 30 minutes before the complement was added. After the complement had been added, and enough salt solution to each tube to bring the total quantity up to 1.25 c.c., the tubes were placed in the incubator at about 37 C., for 1 hour.

Human corpuscles from nonsyphilitic persons, well washed, were used in doses of 0.25 c.c. of a 2.5% suspension in physiologic salt solution. The corpuscles were sensitized for 30 minutes before they were added to the serum-complement-antigen mixture.

The technic used in this investigation differed slightly from that described in previous reports. Of serum, complement, and antigen, one half of the previous quantities were used; of corpuscles, only one fourth, and of hemolytic amboceptor, enough to dissolve this small test dose of corpuscles. Before testing, the human serums were heated to about 55.5 C., for 30 minutes, and 0.6 c.c. of serum-glycerol mixture was diluted to 1.5 c.c. with physiologic salt solution (0.9 gm. of pure sodium chlorid in 1 liter of distilled water). Six

* Received for publicaction July 9, 1917.

[1] Philippine Jour. Sc., Sect. B, 1916, 11, p. 87.

tubes, 1, 2, and 3 as antigen tubes and 1′, 2′, and 3′ as control tubes, were used in each test. Each tube received 0.25 c.c. of diluted serum. Of the 1st pair, Tubes 1 and 1′, each received 0.25 c.c. of 1 : 5 dilution of complement serum; each of the 2nd pair, Tubes 2 and 2′, received 0.25 c.c. of 1 : 10 dilution of complement serum; and each of the 3rd pair, Tubes 3 and 3′, received 0.25 c.c. of 1 : 20 dilution of complement serum. Each of the antigen tubes, Tubes 1, 2, and 3, received a test dose of antigen; and the control tubes, Tubes 1′, 2′ and 3′, received 0.25 c.c. of salt solution each. These mixtures were placed in the incubator at 37 C., for 1 hour. After having been in the incubator 1 hour, each tube received 0.5 c.c. of sensitized corpuscles, representing 0.25 c.c. of 2.5% suspension of washed corpuscles and 1 or 1¼ unit of hemolytic amboceptor. After shaking, the tubes were placed in the incubator at 37 C. for 1 hour; during this time and during the 30 minutes while the corpuscles were being sensitized, the mixture were repeatedly shaken to prevent the corpuscles from settling to the bottom. After having been in the incubator for 1 hour, the tubes were allowed to stand at room temperature for 2 hours, after which the results were read and recorded.

In the antigen control, 6 test tubes were used as in the test, but the human serum was omitted, and the volume was made up with physiologic salt solution. If there was no anticomplementary action in the antigen control, the dose of antigen was considered suitable. If there was anticomplementary action, the dose of antigen was decreased until it was no longer anticomplementary.

TEST 1.—The serum was drawn off the clot on the day after bleeding, and each serum was divided into 2 portions, A and B. Portion A was tested at once and Portion B, unheated, was mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered test tube. Portion B of each serum was tested on May 20, 1917. Immediately before testing the necessary quantity, 0.6 c.c., was heated to 56 C. for 30 minutes.

TABLE 1*

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED IMMEDIATELE BEFORE TESTING

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4424	11/23/15	A	11/24/15	11/24/15	1	0	0	0	+	±	0	Strongly positive
		B	5/20/17	5/20/17	1.25	+	0	0	+	+	tr	Strongly positive
4425	11/23/15	A	11/24/15	11/24/15	1	+	tr	0	+	+	0	Moderately positive
		B	5/20/17	5/20/17	1.25	+	±	0	+	+	tr	Moderately positive
4427	11/23/15	A	11/24/15	11/24/15	1	0	0	0	+	±	0	Strongly positive
		B	5/20/17	5/20/17	1.25	+	0	0	+	+	tr	Strongly positive
4428	11/23/15	A	11/24/15	11/24/15	1	0	0	0	+	±	0	Strongly positive
		B	5/20/17	5/20/17	1.25	+	0	0	+	+	tr	Strongly positive
4429	11/23/15	A	11/24/15	11/24/15	1	0	0	0	+	+	0	Strongly positive
		B	5/20/17	5/20/17	1.25	±	0	0	+	+	tr	Strongly positive
4430	11/24/15	A	11/25/15	11/25/15	1	+	±	0	+	+	0	Weakly positive
		B	5/20/17	5/20/17	1.25	+	+	0	+	+	tr	Weakly positive
4432	11/24/15	A	11/25/15	11/25/15	1	0	0	0	+	±	0	Strongly positive
		B	5/20/17	5/20/17	1.25	±	0	0	+	+	tr	Strongly positive
4434	11/26/15	A	11/27/15	11/27/15	1	tr	0	0	+	±	0	Strongly positive
		B	5/20/17	5/20/17	1.25	+	0	0	+	+	0	Strongly positive

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* In all the tables + means complete hemolysis; ±, hemolysis between 50% and 100%; tr (trace), hemolysis less than 50%; 0, no hemolysis.

TABLE 2

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED BEFORE BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4437	11/26/15	A	11/27/15	11/27/15	1	0	0	0	+	±	0	Strongly positive
		B	11/27/15	5/20/17	1.25	+	0	0	+	+	±	Strongly positive
4438	11/26/15	A	11/27/15	11/27/15	1	+	0	0	+	+	tr	Strongly positive
		B	11/27/15	5/20/17	1.25	+	tr	0	+	+	±	Strongly positive
4439	11/26/15	A	11/27/15	11/27/15	1	+	±	0	+	+	0	Weakly positive
		B	11/27/15	5/20/17	1.25	+	+	tr	+	+	±	Weakly positive
4440	11/27/15	A	11/28/15	11/28/15	1	tr	0	0	+	+	tr	Strongly positive
		B	11/28/15	5/20/17	1.25	±	0	0	+	+	tr	Strongly positive
4441	11/27/15	A	11/28/15	11/28/15	1	0	0	0	+	+	tr	Strongly positive
		B	11/28/15	5/20/17	1.25	tr	0	0	+	+	tr	Strongly positive
4442	11/27/15	A	11/28/15	11/28/15	1	+	0	0	+	+	tr	Strongly positive
		B	11/28/15	5/20/17	1.25	+	±	0	+	+	±	Strongly positive
4443	11/27/15	A	11/28/15	11/28/15	1	tr	0	0	+	+	0	Strongly positive
		B	11/28/15	5/20/17	1.25	+	±	0	+	+	±	Strongly positive
4444	11/27/15	A	11/28/15	11/28/15	1	+	tr	0	+	±	0	Weakly positive
		B	11/28/15	5/20/17	1.25	+	+	tr	+	+	±	Weakly positive

TABLE 3

WASSERMANN REACTION WITH HUMAN SERUM, HEATED BEFORE BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4446	11/30/15	A	12/1/15	12/ 1/15	1	+	0	0	+	+	tr	Strongly positive
		B	12/1/15	5/26/17	1.25	+	tr	0	+	+	tr	Strongly positive
4447	11/30/15	A	12/1/15	12/ 1/15	1	0	0	0	+	+	0	Strongly positive
		B	12/1/15	5/26/17	1.25	0	0	0	+	+	0	Strongly positive
4448	11/30/15	A	12/1/15	12/ 1/15	1	+	tr	0	+	+	tr	Strongly positive
		B	12/1/15	5/26/17	1.25	+	tr	0	+	+	tr	Strongly positive
4449	11/30/15	A	12/1/15	12/ 1/15	1	0	0	0	+	+	tr	Strongly positive
		B	12/1/15	5/26/17	1.25	0	0	0	+	+	0	Strongly positive

TABLE 4

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED IMMEDIATELY BEFORE TESTING

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4452	12/1/15	A	12/ 2/15	12/ 2/15	1	tr	0	0	+	+	tr	Strongly positive
		B	5/26/17	5/26/17	1.25	tr	0	0	+	+	0	Strongly positive
4453	12/1/15	A	12/ 2/15	12/ 2/15	1	+	0	0	+	±	0	Moderately positive
		B	5/26/17	5/26/17	1.25	0	0	0	tr	0	0	Anticomplementary
4454	12/1/15	A	12/ 2/15	12/ 2/15	1	tr	0	0	+	+	tr	Strongly positive
		B	5/26/17	5/26/17	1.25	+	0	0	+	+	tr	Strongly positive
4456	12/1/15	A	12/ 2/15	12/ 2/15	1	0	0	0	+	+	tr	Strongly positive
		B	5/26/17	5/26/17	1.25	0	0	0	+	±	0	Strongly positive

Table 1 shows that 8 serums gave practically the same results on May 20, 1917, as they did in November, 1915. None of them had become permanently anticomplementary.

TEST 2.—The serum was drawn off the clot the day after the bleeding, and each serum was divided into 2 portions, A and B. Portion A was tested immediately and Portion B was heated to about 55.5 C. for 30 minutes, mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered test tube. Without having been reheated, Portion B was tested on May 20, 1917.

Table 2 shows that the results obtained on May 20, 1917, were practically identical with the results obtained in November, 1915. The serums had not become anticomplementary and remained clear.

TEST 3.—The serum was drawn off the clots on the day after the bleeding, and each serum was divided into 2 portions, A and B. Portion A was tested the next day, and Portion B was heated to 55.5 C. for 30 minutes, and mixed with an equal volume of sterilized, chemically pure glycerol, placed in cold storage at about 7 C. until the latter part of April, 1916; then it was kept at room temperature in a cork-stoppered test tube.

Without having been reheated, Portion B was tested on May 26, 1917.

Table 3 shows that the results obtained were practically the same on May 26, 1917, as on Dec. 1, 1915. None of the serum had become anticomplementary or cloudy.

TEST 4.—The serum was drawn off the clot on the day after bleeding. Each serum was divided into 2 portions. Portion A was tested at once, and Portion B was mixed with an equal volume of sterilized, chemically pure glycerol, kept in the cold storage at about 7 C. until the latter part of April, 1916, then at room temperature. On May 26, 1917, the necessary quantity was heated to about 56 C. for 30 minutes and tested.

Table 4 shows that the results obtained on May 26, 1917, nearly correspond to those of Dec. 2, 1915. Serum 4453 had become anticomplementary.

TEST 5.—The serum was drawn off the clot on the day after bleeding. Each serum was divided into 2 portions. Portion A was tested at once, and Portion B was mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered tube. On May 27, 1917, the necessary quantity was heated to about 56 C. for 30 minutes and tested.

Table 5 shows that the results obtained with Serums 4535, 4536, 4537, and 4538 on May 27, 1917, were identical with the results obtained on Dec. 28, 1915. Serum 4538 had become moderately anticomplementary.

TEST 6.—The serum was drawn off the clot the day after bleeding. Each serum was divided into 2 portions, A and B. Portion A was tested at once, and Portion B was heated to about 55.5 C. for 30 minutes, mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered tube.

On May 27, 1917, Portion B was tested without having been reheated.

The results obtained on May 27, 1917, are identical with those obtained on Dec. 28, 1915. None of these serums had become anticomplementary.

TABLE 5

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED IMMEDIATELY BEFORE TESTING

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4535	12/27/15	A	12/28/15	12/28/15	1	+	+	tr	+	+	tr	Negative
		B	5/27/17	5/27/17	1.25	+	+	0	+	+	0	Negative
4536	12/27/15	A	12/28/15	12/28/15	1	+	tr	0	+	tr	0	Negative
		B	5/27/17	5/27/17	1.25	+	+	0	+	+	0	Negative
4537	12/27/15	A	12/28/15	12/28/15	1	+	+	0	+	+	0	Negative
		B	5/27/17	5/27/17	1.25	+	+	0	+	+	0	Negative
4538	12/27/15	A	12/28/15	12/28/15	1	+	+	0	+	+	0	Negative
		B	5/27/17	5/27/17	1.25	+	0	0	+	0	0	Negative

TABLE 6

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED BEFORE BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4540	12/27/15	A	12/28/15	12/28/15	1	+	+	tr	+	+	tr	Negative
		B	12/28/15	5/27/17	1.25	+	+	0	+	+	0	Negative
4541	12/27/15	A	12/28/15	12/28/15	1	+	+	tr	+	+	tr	Negative
		B	12/28/15	5/27/17	1.25	+	+	0	+	+	0	Negative
4542	12/27/15	A	12/28/15	12/28/15	1	+	+	tr	+	+	tr	Negative
		B	12/28/15	5/27/17	1.25	+	+	0	+	+	0	Negative
4544	12/27/15	A	12/28/15	12/28/15	1	+	+	0	+	+	0	Negative
		B	12/28/15	5/27/17	1.25	+	+	0	+	+	0	Negative

TEST 7.—The serum was drawn off the clot on the day after bleeding. Each serum was divided into 2 portions, A and B. Portion A was tested immediately and Portion B, unheated, was mixed with an equal volume of sterilized, chemically pure glycerol, and kept in a cork-stoppered tube in cold storage until the latter part of April, 1916; then it was kept at room temperature.

On May 27, 1917, the necessary quantity was heated to 56 C. for 30 minutes and tested.

As Table 7 shows, 3 of the serums had become anticomplementary.

TEST 8.—The serums were drawn off the clot on the day after bleeding. Each serum was divided into 2 portions, A and B. Portion A was tested at once. Portion B was heated to about 55.5 C. for 30 minutes, mixed with an equal volume of sterilized, chemically pure glycerol, and kept in a cork-stoppered tube in cold storage at about 7 C. until the latter part of April, 1916; then it was kept at room temperature. On May 27, 1917, Portion B was tested.

The results, on May 27, 1917, are the same as on Dec. 30, 1915. No serum had become anticomplementary.

TEST 9.—About 24 hours after having been secured, the serum was drawn off the clot. Each serum was divided into 2 portions, A and B. Portion A was tested immediately. Portion B was heated to about 55.5 C. for 30 minutes, mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered test tube. On June 3, 1917, Portion B. was tested.

TABLE 7

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED IMMEDIATELY BEFORE TESTING

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4546	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	5/27/17	5/27/17	1.25	+	+	0	+	+	0	Negative
4548	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	5/27/17	5/27/17	1.25	0	0	0	0	0	0	Anticomplementary
4549	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	5/27/17	5/27/17	1.25	tr	0	0	tr	0	0	Anticomplementary
4550	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	5/27/17	5/27/17	1.25	+	tr	0	+	tr	0	Negative

TABLE 8

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED BEFORE BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4551	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	12/30/15	5/27/17	1.25	+	+	0	+	+	0	Negative
4553	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	12/30/15	5/27/17	1.25	+	+	tr	+	+	tr	Negative
4554	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	12/30/15	5/27/17	1.25	+	+	tr	+	+	tr	Negative
4555	12/29/15	A	12/30/15	12/30/15	1	+	+	tr	+	+	tr	Negative
		B	12/30/15	5/27/17	1.25	+	+	0	+	+	0	Negative

TABLE 9

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED BEFORE BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Tested	Amboceptor Unit	Readings						Result
						Antigen Tubes			Control Tubes			
						1	2	3	1′	2′	3′	
4662	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	±	+	+	±	Negative
		B	4/ 6/16	6/ 3/17	1.25	+	+	0	+	+	0	Negative
4664	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	±	+	+	±	Negative
		B	4/ 6/16	6/ 3/17	1.25	+	+	0	+	+	0	Negative
4667	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	±	+	+	±	Negative
		B	4/ 6/16	6/ 3/17	1.25	+	+	0	+	+	0	Negative
4669	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	±	+	+	±	Negative
		B	4/ 6/16	6/ 3/17	1.25	+	+	0	+	+	0	Negative
4673	4/ 7/16	A	4/ 8/16	4/ 8/16	1	+	+	±	+	+	±	Negative
		B	4/ 8/16	6/ 3/17	1.25	+	+	0	+	+	0	Negative
4684	4/ 9/16	A	4/10/16	4/10/16	1	+	+	±	+	+	±	Negative
		B	4/10/16	6/ 3/17	1.25	+	+	tr	+	+	tr	Negative
4650	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	tr	+	+	±	Weakly positive
		B	4/ 6/16	6/ 3/17	1.25	+	tr	0	+	+	0	Weakly positive
4652	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	+	tr	+	+	±	Weakly positive
		B	4/ 6/16	6/ 3/17	1.25	+	tr	0	+	+	0	Moderately positive
4707	4/11/16	A	4/12/16	4/12/16	1	+	+	tr	+	+	±	Weakly positive
		B	4/12/16	6/ 3/17	1.25	+	tr	0	+	+	0	Moderately positive
4657	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	±	0	+	+	±	Strongly positive
		B	4/ 6/16	6/ 3/17	1.25	+	0	0	+	+	0	Strongly positive
4668	4/ 5/16	A	4/ 6/16	4/ 6/16	1	+	±	0	+	+	±	Strongly positive
		B	4/ 6/16	6/ 3/17	1.25	+	0	0	+	+	0	Strongly positive
4653	4/ 5/16	A	4/ 6/16	4/ 6/16	1	0	0	0	+	+	±	Strongly positive
		B	4/ 6/16	6/ 3/17	1.25	0	0	0	+	+	0	Strongly positive

As Table 9 shows, the results obtained on June 3, 1917, were nearly identical with the results in April, 1916. Three serums, 4650, 4652, and 4707 reacted a little stronger than they did in April, 1916. None of the 12 serums had become anticomplementary.

TEST 10.—The serum was drawn off the clot about 24 hours after the bleeding. Each serum was divided into 2 portions, A and B. Portion A was tested immediately; Portion B was mixed with an equal volume of sterilized, chemically pure glycerol, and kept at room temperature in a cork-stoppered test tube. Seven days after having been mixed with glycerol, each serum was heated to about 55.5 C. for 30 minutes. After having been heated the serum was kept at room temperature.

On June 9, 1917, Portion B was tested before and after having been reheated.

Table 10 shows that the results obtained on June 9, 1917, were practically identical with the results in February, 1916. The serums had become slightly anticomplementary. The anticomplementary property was completely destroyed by heating to 56 C. for 30 minutes.

TABLE 10

WASSERMANN REACTION WITH GLYCEROLATED HUMAN SERUM, HEATED 7 DAYS AFTER BEING MIXED WITH GLYCEROL

Number	Date of Bleeding	Portions (A, Nonglycerolated; B, Glycerolated)	Heated	Reheated	Tested	Amboceptor Unit	Readings						Result
							Antigen Tubes			Control Tubes			
							1	2	3	1′	2′	3′	
4618	2/25/16	A	2/26/16		2/26/16	1	+	+	tr	+	+	tr	Negative
		B	3/ 4/16		6/ 9/17	1.25	+	tr	0	+	tr	0	Negative
		B	3/ 4/16	6/ 9/17	6/ 9/17	1.25	+	+	tr	+	+	tr	Negative
4621	2/26/16	A	2/27/16		2/27/16	1	+	0	0	+	+	tr	Strongly positive
		B	3/ 5/16		6/ 9/17	1.25	+	0	0	+	+	0	Strongly positive
		B	3/ 5/16	6/ 9/17	6/ 9/17	1.25	+	0	0	+	+	tr	Strongly positive
4616	2/24/16	A	2/25/16		2/25/16	1	+	0	0	+	+	tr	Strongly positive
		B	3/ 3/16		6/ 9/17	1.25	tr	0	0	+	tr	0	Strongly positive
		B	3/ 3/16	6/ 9/17	6/ 9/17	1.25	+	0	0	+	+	tr	Strongly positive
4617	2/24/16	A	2/25/16		2/25/16	1	0	0	0	+	+	tr	Strongly positive
		B	3/ 3/16		6/ 9/17	1.25	0	0	0	+	±	0	Strongly positive
		B	3/ 3/16	6/ 9/17	6/ 9/17	1.25	±	0	0	+	+	tr	Strongly positive

CONCLUSIONS

Pure glycerol is an ideal preservative for human serum intended for the Wassermann reaction. In order to prevent the serum from becoming anticomplementary, it must be heated to about 56 C. for 30 minutes and then be mixed with an equal volume of glycerol. Serum kept for more than a year gave practically the same results as when fresh and without preservative.